FAILURE OF PINEALECTOMY TO ALTER GONADAL ACTIVITY IN HARRIS' SPARROW (ZONOTRICHIA QUERULA) AND THE TREE SPARROW (SPIZELLA ARBOREA)

by

RICHARD S. DONHAM

B. S., Kansas State University, 1965

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

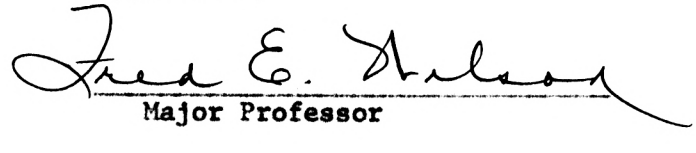
MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Approved by



Major Professor

TABLE OF CONTENTS

INTRODUCTION

The role of the pineal body or epiphysis cerebri in mammalian and avian reproduction is unclear (for reviews, see Andersen and Wolf, 1934; Kitay and Altschule, 1954). While many investigators find that pinealectomy results in gonadal stimulation (Foà, 1912, 1914; Horrax, 1916; Izawa, 1922, 1923, 1926; Renton and Rusbridge, 1932; Simonnet and Thieblot, 1951; Kitay, 1954; Wurtman et al., 1959, 1961; Ifft, 1962; Gittes and Chu, 1965; Hoffman and Reiter, 1965a,b, 1966; Roth, 1965; Reiter et al., 1966a,b), others find that it inhibits gonadal growth, even in the same species (Shellabarger, 1952; Sayler and Wolfson, 1967). Still other investigators find that pinealectomy neither stimulates nor inhibits gonadal function (Badertscher, 1924; del Castillo, 1928; Andersen and Wolf, 1934; Stalsberg, 1965; Kincl and Benagiano, 1967; Wragg, 1967). Many of these seemingly contradictory results are not entirely inconsistent since some evidence indicates that the effect of the pineal is transient (Shellabarger and Breneman, 1949; Arrington, 1966; Homma et al., 1967; Sayler and Wolfson, 1967) and sex-dependent (Foà, 1912, 1914; Horrax, 1916; Homma et al., 1967; Sayler and Wolfson, 1967).

Results of replacement studies are more consistent than those of ablation studies. Injection of pineal extracts (Wurtman et al., 1959, 1961; Meyer et al., 1961; Moszkowska, 1963; Reiss et al., 1963; Homma et al., 1967) or transplantation of the pineal to the kidney capsule or muscle (Gittes and Chu, 1965) or to the anterior chamber of the eye (Simonnet and Thieblot, 1951) usually inhibits gonadal development. A number of indole derivatives has been isolated from pineal tissue (see

Quay, 1965, for review). One of these, N-acetyl-5-methoxytryptamine (melatonin), first isolated by Lerner et al. (1958), inhibits ovarian development in rats exposed to constant light (Wurtman et al., 1963; Wurtman and Axelrod, 1965). It should be emphasized, however, that this compound does not inhibit gonadal development in golden hamsters (Reiter et al., 1966a) in which reproduction is influenced strongly by the pineal (Hoffman and Reiter, 1965a,b, 1966; Reiter and Hester, 1966; Reiter et al., 1966a,b; Reiter, 1967). Apparently, then, the inhibitory action of the pineal is not always mediated by melatonin.

Obviously, there is no unanimity of opinion as to what effect the pineal exerts or if it affects reproduction at all. However, as Kitay and Altschule (1954) note, a majority of the literature favors an hypothesis that assigns an inhibitory function to the pineal.

The rat and chicken have been the most commonly utilized laboratory animals in pineal-gonadal studies. Since these animals have been inbred through many generations for maximal breeding performance, their propensity for maintaining functional gonads may mask subtle influences of the pineal that might modify reproduction in less domesticated species. The pineal conceivably could modify reproduction more strongly in seasonal breeders than in laboratory animals. Pineal ablation in seasonal breeders might be reflected by conspicuous alterations in cyclic reproductive activity.

Harris' sparrow (Zonotrichia querula) and the tree sparrow (Spizella arborea), like other passerine birds, have annual reproductive cycles. Definitive studies on the gonadal cycles of these sparrows are lacking, but migration and molting observations are available (Swenk and Stevens, 1929; Harkins, 1937; Stevens, 1957). Vernal migration and molting, like

gonadal growth and development, occur annually at times typical for migratory passerine species. In addition, gonadal growth can be induced photoperiodically in both species (Wilson, 1968; Wilson and Hands, 1968; Wilson and Morrison, unpublished). Moreover, gonadal activity can be predicted in the sham-operated controls in this investigation (see Results). Considerable circumstantial evidence, therefore, suggests that Harris' and tree sparrows exhibit annual gonadal cycles typical of those of many North Temperate Zone migrants.

Such a gonadal cycle is typified by that of the white-crowned sparrow (Zonotrichia leucophrys gambelii) (Fig. 1; Blanchard and Erickson, 1949; King et al., 1966). Following a period of reproductive quiescence, the longer day lengths of winter and spring induce gonadal development. In males, at least, no other environmental factors are required since exposure of photosensitive birds to long daily photoperiods in the laboratory invariably induces maximal testicular development. After an interval of maximal testicular size and activity, photostimulation will no longer maintain functional testes and they regress to the minimal size characteristic of migratory passerine species in late summer and fall. Thus, a refractoriness to photostimulation develops that is accompanied by involution of the testes. Termination of refractoriness requires a period of retention on short days. By proper manipulation of the daily photoperiod, events of the testicular cycle can be simulated in the laboratory. Certain ecological factors in addition to photostimulation are required to induce full ovarian development; otherwise, the ovarian cycle parallels the testicular cycle.

The role of the pineal in cyclic reproduction of avian species is suggested both by the evidence for a transient function of the pineal

Fig. 1. Testicular and ovarian cycles of the white-crowned sparrow (Zonotrichia leucophrys gambelii). These gonadal cycles are typical of those of many other North Temperate Zone passerines. Closed circle represents testes; open circle represents ovaries. The scale at the top indicates periods of the gonadal cycle. Functional state of Harris' or tree sparrows at time of pinealectomy or sham operation for each of the experiments is indicated by the arrows. (Modified from King et al., 1966).

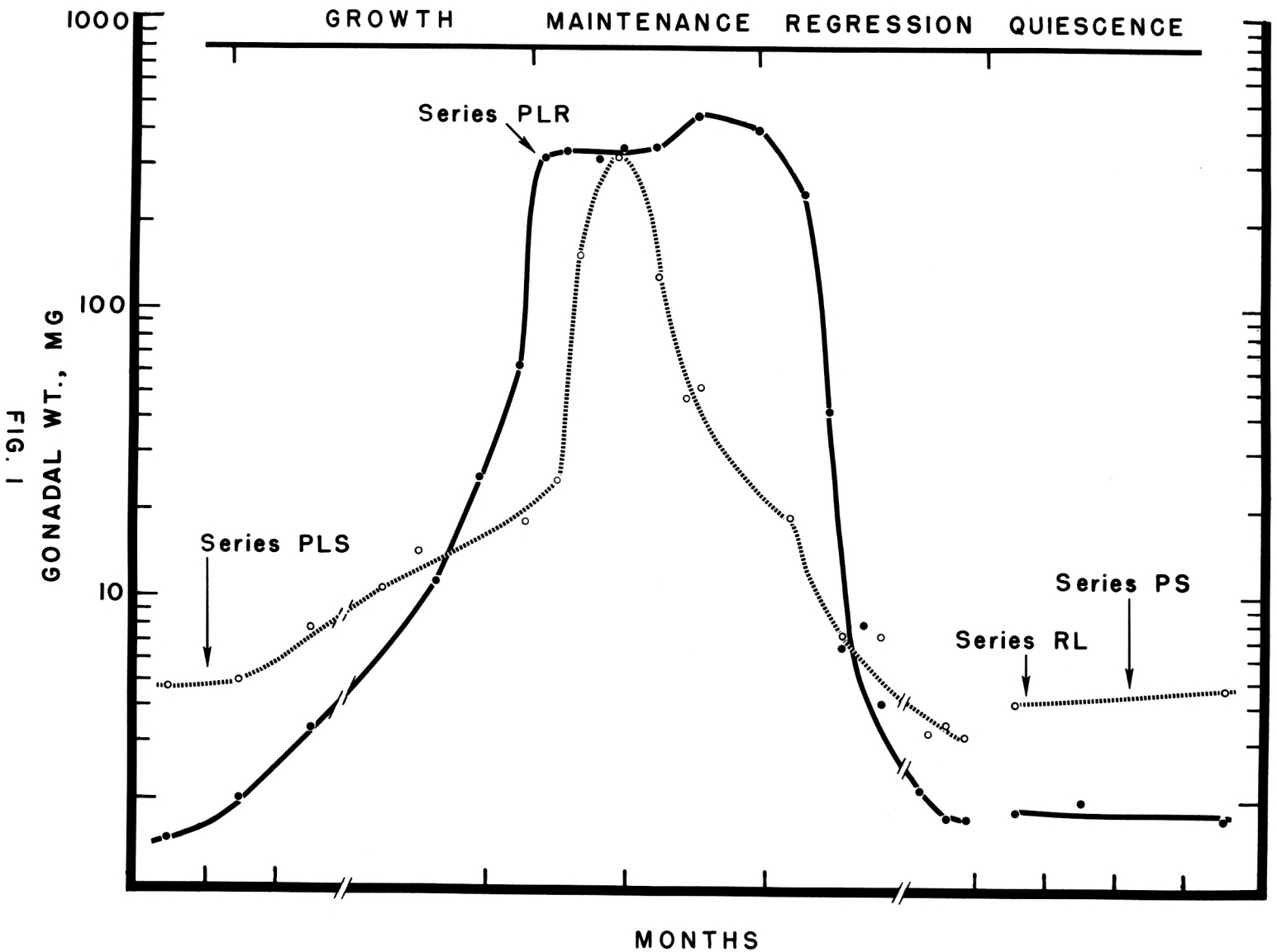

GROWTH
MAINTENANCE
REGRESSION
QUIESCENCE
1000
100
10
GONADAL WT., MG
Series PLR
Series PLS
Series PS
Series RL
MONTHS

FIG. 1

(Shellabarger and Breneman, 1949; Arrington, 1966; Homma et al., 1967; Sayler and Wolfson, 1967) and by the evidence that, although the etiology of refractoriness is unknown, refractoriness is endogenous (see Farner, 1959, for review). This evidence, and the probability that an antigonadotropic function exists in some species, indicates that studies that determine the pineal-gonadal relationship, especially during refractoriness and gonadal regression, would be pertinent. This investigation comprises a series of tests of the hypothesis that the pineal has an antigonadotropic function in Harris' sparrow and the tree sparrow.

THE EXPERIMENTS

If the pineal has a role in the avian reproductive cycle, that role should be indicated by subjecting pinealectomized birds to a variety of photoperiodic treatments. Individual experiments in this study determine the effect of pinealectomy on gonadal weight and histological architecture during one or more phases of the gonadal cycle: regression, quiescence due to nonphotostimulation, and quiescence due to refractoriness. For each series of experiments, time of pinealectomy with respect to gonadal cycle is shown in Fig. 1.

Series PS

Male and female Harris' sparrows captured from 17 December 1966 - 4 February 1967 were retained on 8-hr daily photoperiods in the laboratory (Table 1). They were pinealectomized or sham-pinealectomized in mid-July and killed 1.5 - 3 months later. Intact males and females were killed on the same day that operations were performed on experimental animals. The purpose of this experiment was to determine the role of the pineal during gonadal quiescence in photosensitive, nonphotostimulated birds.

Series PLR

Male Harris' sparrows captured from 17 December 1966 - 4 February 1967 were held on 8-hr daily photoperiods until exposure to the experimental lighting regimen (Table 1). Testicular development (observed by periodic laparotomy) was induced by lengthening the daily photoperiod to 20 hrs on 27 February 1967. Twenty days later, i.e., near the end of the logarithmic growth phase (Wilson, 1968), operations were performed and the birds

Table 1. Experimental treatment of Harris' sparrows.

Group[a]	Sex	Number and treatment of birds[b]	Photoperiod prior to experiment	Experimental photoperiod	Time from operation to killing
PS controls	M	5	8L:16D		
PS_1	M	8P 9S	8L:16D	8L:16D	2 months
PS controls	F	5	8L:16D		
PS_2	F	6P 5S	8L:16D	8L:16D	1.5 months
PS_3	F	6P 6S	8L:16D	8L:16D	3 months
PLR_1	M	4P 5S	8L:16D	20L:4D	54 days[c]
PLR_2	M	4P 6S	8L:16D	20L:4D	79 days[c]
RL_1	M	7P 2S	13L:11D to 20L:4D[d]	20L:4D	1.5 months
RL_2	M	5P 4S	13L:11D to 20L:4D[d]	20L:4D	3 months
RL_3	F	12P 5S	13L:11D to 20L:4D[d]	20L:4D	1 month

RL_4	F	10P 6S	13L:11D to 20L:4D[d]	20L:4D	2 months
RL_5	F	10P 6S	13L:11D to 20L:4D[d]	20L:4D	3 months

[a] Subscripts refer to group number. PS controls, birds sacrificed when PS birds were pinealectomized or sham operated; PS, photosensitive, nonphotostimulated birds; PLR, photosensitive, photostimulated birds that became refractory during the experiment; RL, refractory, photostimulated birds.

[b] P, pinealectomy; S, sham operation.

[c] Pinealectomized or sham operated after 20 days' exposure to 20-hr daily photoperiods.

[d] Two to six weeks on 13L:11D; then to 20L:4D for at least five months to maintain refractoriness.

returned to long days. One group was killed during gonadal involution (54 days postoperatively); another was allowed to complete involution (killed 79 days postoperatively). This experiment was designed to study the influence of the pineal during gonadal regression induced by continued photostimulation.

Series RL

Male and female Harris' sparrows captured from 12 March - 16 April 1966 were kept initially on 13-hr days (Table 1). The daily photoperiod was increased to 20 hrs on 2 May 1966 to accelerate completion of gonadal development. About five months later (after the induction of refractoriness), operations were performed and the birds returned to long days. Birds were killed one to three months after operation. This experiment was designed to study the influence of the pineal on the gonads during the refractory period.

Series PLS

Male tree sparrows captured from 3 January - 4 February 1967 were held on 8-hr daily photoperiods until the experiment began (Table 2). Operations were performed a few days prior to transfer to 20-hr daily photoperiods on 22 April 1967. After testicular development was essentially complete (34 days after initiating long days), the birds were transferred to 2-hr daily photoperiods. Birds were sacrificed 15 or 30 days thereafter. Series PLS is similar to Series PLR except that gonadal regression was induced by short days rather than by long days. This experiment, like that of Series PLR, was designed to study the role of the pineal during induction of testicular regression.

Table 2. Experimental treatment of tree sparrows.

Group[a]	Sex	Number and treatment[b] of birds	Photoperiod prior to experiment	Experimental photoperiod
PLS_1	M	4P 4S	8L:16D	20L:4D to 2L:22D[c]
PLS_2	M	3P 5S	8L:16D	20L:4D to 2L:22D[d]

[a] Subscripts refer to group number. PLS, photosensitive, photostimulated birds that were transferred to 2-hr daily photoperiods during the experiment.
[b] Designation as in Table 1.
[c] 20L:4D for 34 days; then 2L:22D for 15 days.
[d] 20L:4D for 34 days; then 2L:22D for 30 days.

MATERIALS AND METHODS

Capture and Maintenance

The birds used in this investigation were captured with mist nets from wintering populations near Manhattan, Kansas. Prior to the experiment, some birds were retained in windowless aviaries; during the experiment, all were held, two to five per cage, in small cages (ca. 23 x 25 x 41 cm or 25 x 25 x 51 cm). Experimental lighting was provided by fluorescent, or by both fluorescent and incandescent, lamps. Minimum illumination for birds on long daily photoperiods was 375 lux. Food (a vitamin- and mineral-enriched chick-starter crumble supplemented with commercially prepared parakeet foods) and water were freely available. Ambient temperature varied between 18° and 24° C.

At the end of each experiment, birds were killed by decapitation. Gonads were fixed for five days in acetic acid, formalin, and ethanol (Farner et al., 1966); after five additional days in 70% ethanol, they were debrided of extraneous tissue and weighed to the nearest 0.01 mg on a torsion balance. Brains were fixed for 24 to 48 hours in Bouin's fluid while the region of the pineal was carefully dissected. Gonads and the prepared region of the pineal were embedded in Paraplast through methylbenzoate-benzene, sectioned sagittally at 10μ, and stained with hematoxylin-eosin. Operational success was verified microscopically: only fully pinealectomized or sham-operated birds with intact pineals were considered in the results. Testicular development was assessed microscopically using the index of Bartholomew (1949). The stages of this index are:

I. resting spermatogonia only

II. spermatogonia dividing, but only a few spermatocytes present

III. spermatocytes

IV. spermatocytes with spermatids

V. spermatids with a few sperm

VI. full spermatogenic activity with many sperm

An additional stage was necessary to characterize regressing gonads:

R. moderate to complete involution of all tubular constituents except spermatogonia

Statistical analysis of gonadal and body weights was by Student's *t*-test.

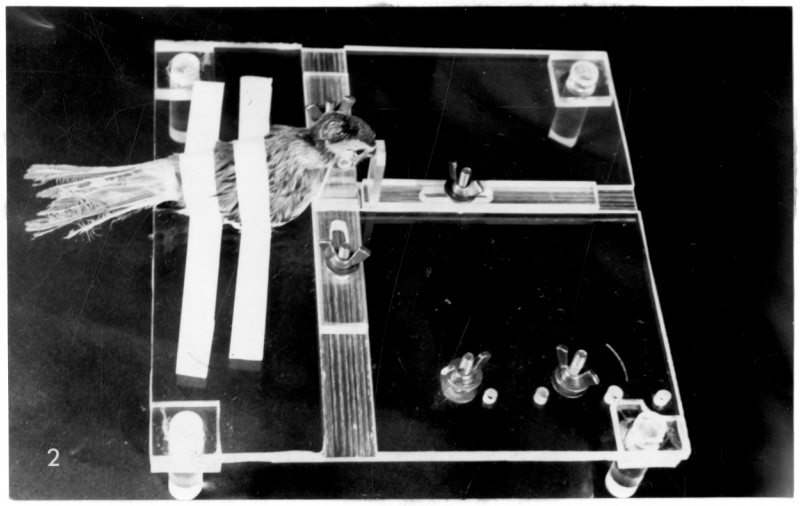
Operative Procedure

The pineal gland in avian species is a stalked organ that arises from the roof of the diencephalon and projects dorsocaudad between the cerebellum and cerebral hemispheres (Tilney and Warren, 1919; Krabbe, 1955). It is posterior to, and intimately associated with, the union of the longitudinal and transverse sinuses and inferior and caudal to the junction of the longitudinal and transverse sutures of the cranium. It is a consolidated organ encapsulated by connective tissue which is continuous with that of the dorsal meninges.

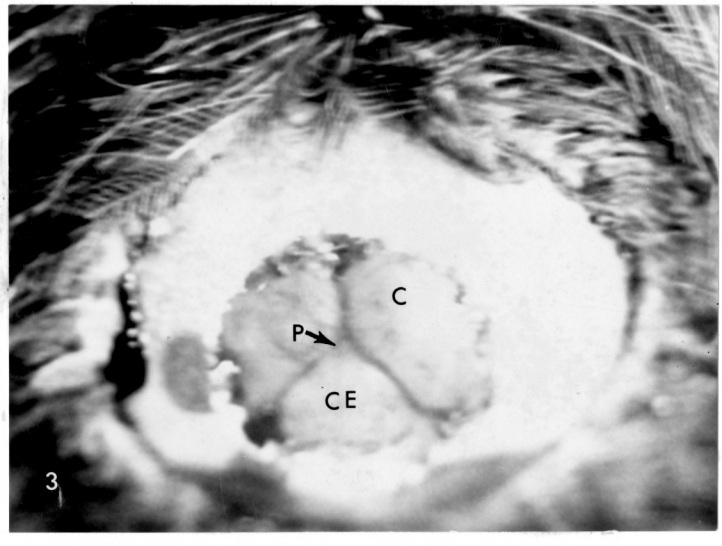
There are numerous descriptions of pinealectomy in avian species in the literature (Foà, 1912; Izawa, 1922; Shellabarger and Breneman, 1949; Stalsberg, 1965; Homma *et al.*, 1967). All of these techniques are similar; the following modification is not necessarily superior, but one that worked well in Harris' and tree sparrows. Immobilizing the bird's head in a stereotaxic apparatus designed for passerine species (Wilson, 1965) greatly facilitated the operation (Figs. 2 and 3).

Fig. 2. A tree sparrow prepared for pinealectomy. The sparrow's head is immobilized and body movement is prevented by strips of masking tape. About ½ natural size.

Fig. 3. Dorsal view of the head of a tree sparrow prepared for pinealectomy. The head has been immobilized in the base of the stereotaxic device. The incised skin was retracted, exposing the cranium in the region of the junction of the longitudinal and transverse sutures, and then a circular patch of the skull over the pineal was removed. P pineal body; CE cerebellum; C cerebrum. About X 8



2



3

After pentobarbital sodium anesthesia (Donovan, 1958), the bird was mounted in the stereotaxic device and the feathers on the back and crown of the head were trimmed to the skin. The junction of the longitudinal and transverse sutures of the cranium was exposed by retracting the overlying skin after it had been incised. The bone of the skull in these birds consists of an outer laminar layer, a middle reticular layer, and an inner laminar layer; often the pineal could be more easily seen through these layers by clearing the bone with a drop of mineral oil. The rest of the surgery was performed under a dissecting microscope. Using a sharp, bent-tip probe, a circle of about 0.5 cm was punctured in the outer layer of bone over the pineal. This plate was lifted off, the reticular layer cleared away with fine forceps, and the inner plate punctured with the probe. When the latter layer was carefully removed, the meninges overlying the pineal remained intact. A transverse incision in the dura mater was made caudad to the pineal. If the pineal was grasped with forceps, it often fragmented as it was removed. However, since the gland is quite firmly attached to the dura, it usually could be removed intact by grasping the dura posterior to the pineal and lifting forward. Placing the pineal in Bodian's fixative (see Ziesmer, 1951) caused the gland to become quite opaque; it was inspected to verify completeness of removal. Application of Surgicel (Johnson & Johnson, New Brunswick, New Jersey) or Gelfoam (Upjohn, Kalamazoo, Michigan) usually controlled any hemorrhage. After stopping the hemorrhage, the skin was replaced. Sham operation was performed in the manner described above, except that the pineal was left intact. Since pinealectomy was almost always followed by bleeding from one or more of the sinuses near the gland, sham operation included rupture of at least one of these sinuses. Even so, bleeding

from sham operation was usually not as severe as from pinealectomy.

RESULTS

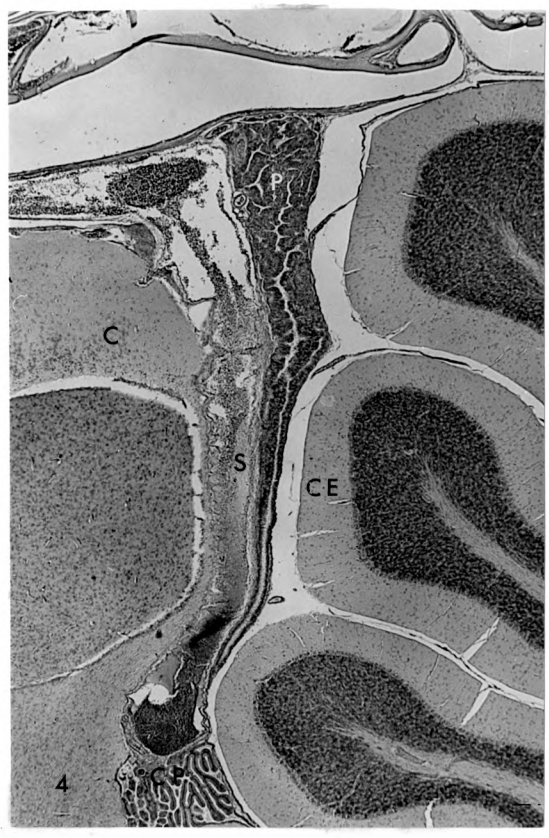
As noted previously, the pineal body of birds and mammals is a derivative of the posterior roof of the diencephalon. In both Harris' and tree sparrows, it is club-shaped or somewhat triangular; a slender stalk expands distally to form the body of the pineal (Fig. 4). In Zonotrichia querula, the maximum width of 16 pineals ranged from 0.16-0.80 mm with a mean of 0.39 mm. Pineal length ranged from 0.90-2.78 mm with a mean of 1.86 mm. Position, size, and shape of the pineal appear to depend on the proximity of adjacent blood vessels and brain masses (cerebrum and cerebellum). Connective tissue septae penetrate and ramify throughout the pineal; these provide a supporting framework that divides the richly vascularized gland into lobules. In the pineal there is a continuous central lumen with many diverticula and follicular extensions (Fig. 4). As the lumen extends into the proximal portion of the pineal stalk, it decreases in size. In the most proximal portion of the stalk, the lumen is absent or obliterated.

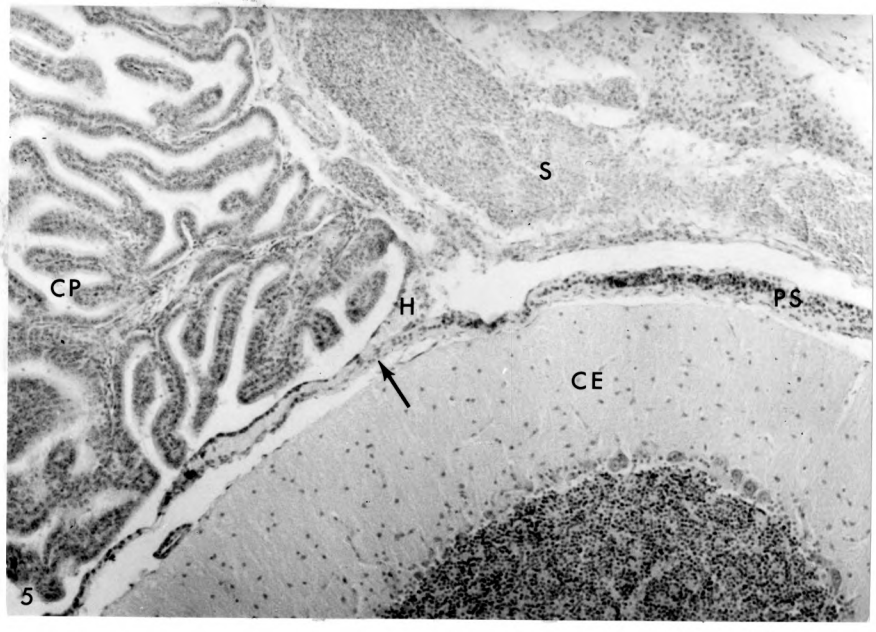
In adult Z. querula it is difficult to define the point of attachment of the pineal to the diencephalon. In most specimens, the pineal stalk appears to arise from epithelium of the dorsal sac, an expansion of the diencephalic roof immediately anterior to the most proximal portion of the pineal stalk. The avian pineal gland is generally considered to originate between the habenular and posterior commissures (Quay and Renzoni, 1967). Among the brains examined here, only one pineal stalk clearly extends beyond the dorsal sac to the region of the habenular commissure (Fig. 5).

The bulk of the pineal is composed of parenchymal cells of neuro-

Fig. 4. Region of the pineal of a sham-operated Harris' sparrow. The pineal body (P) is bounded anteriorly by the cerebrum (C), posteriorly by the cerebellum (CE), dorsally by the meninges and skull, and ventrally by the choroid plexus (CP). S venous sinus. Zq0294, PS_2. Bouin. Sagittal section. Hematoxylin-eosin. About X 40

Fig. 5. Region of the pineal stalk of a sham-operated Harris' sparrow showing the anatomical relationship of the pineal stalk (PS) to the habenular commissure (H). Arrow denotes the area of the attachment of the pineal to the diencephalic roof of the third ventricle. CP choroid plexus of the roof of the third ventricle; CE cerebellum; S venous sinus. Zq0226, PLR_1. Bouin. Sagittal section. Hematoxylin-eosin. X 124


P
C
S
CE
4
CP


S
CP
H
PS
CE
5

ectodermal origin. These cells have a diameter of about 5-15μ. The nucleus is large and clearly defined; except for one or more intensely basophilic granules, the nucleus is lightly stained (Fig. 6). Nuclear diameter ranges from 4-6μ. In addition to parenchymal cells, nerve fibers are observed in the pineal. Indeed, the proximal pineal stalk is a bundle of nerve fibers with a few scattered glial and supportive cells; the parenchymal component and central lumen are absent. Anomalous nodular structures are occasionally seen in or near the pineal; these vary in number and in position and do not resemble pineal parenchyma.

Series PS

Sham-operated, photosensitive birds exposed to 8-hr daily photoperiods showed no gonadal development; likewise, photosensitive, nonphotostimulated Harris' sparrows that were pinealectomized demonstrated no gonadal growth (Table 3). Testicular weights were not significantly different two months after pinealectomy than at operation. A histological survey of one PS control, one sham-operated and two pinealectomized birds revealed that all had testes with seminiferous tubules in Stage II of development (Fig. 7). Leydig cells were frequently observed in the interstitium. Ovarian weights of PS controls sacrificed at operation were similar to ovarian weights of pinealectomized and sham-operated birds killed three months later. Ovarian weights of sham-operated birds were heavier at 1.5 months than at 3 months after operation, but never were ovarian weights of sham-operated birds significantly different from those of pinealectomized birds.

Table 3. Failure of pinealectomy to affect gonadal weight and body weight of photosensitive Harris' sparrows exposed to 8-hr daily photoperiods.

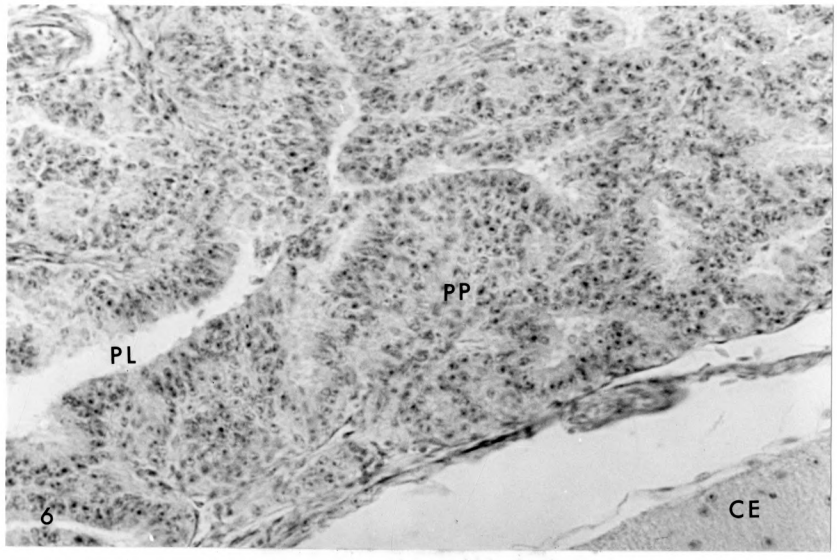
Group[a]	Sex	Operational procedure[b]	Months on 8L:16D after operation[c]	Gonadal weight[d] (mg ± S. E.)	Body weight at sacrifice[e] (gm ± S. E.)
PS controls	M		-0-	3.10 ± 0.631 (5)	34.4 ± 0.67 (5)
PS_1	M	P	2	7.27 ± 1.733 (8)	38.0 ± 0.74 (8)
		S		6.58 ± 1.399 (9)	37.4 ± 1.25 (9)
PS controls	F		-0-	10.20 ± 2.150 (5)	31.8 ± 0.44 (5)
PS_2	F	P	1.5	11.63 ± 1.412 (6)[f]	30.7 ± 0.70 (6)
		S		14.33 ± 2.350 (5)[f]	31.1 ± 0.58 (5)
PS_3	F	P	3	9.92 ± 1.825 (6)	31.7 ± 1.80 (6)
		S		8.84 ± 1.021 (6)	33.1 ± 1.67 (6)

[a] PS controls were killed when the operations were performed; group designations as in Table 1.
[b] Designation as in Table 1.
[c] Birds had been held on 8-hr daily photoperiods 5-7 months prior to operation.
[d] Values for males represent combined testicular weights. Sample males had testes in Stage II of tubular development. Number of birds indicated in parentheses.
[e] Number of birds indicated in parentheses.
[f] Significantly different ($P<0.05$) from sham-operated birds of group PS_3.

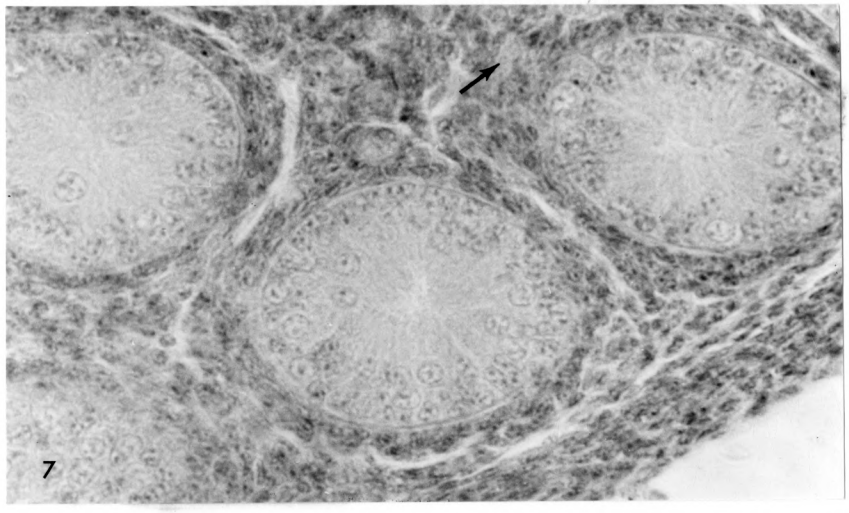
Fig. 6. Pineal parenchyma (PP) of a sham-operated Harris' sparrow. PL lumen of the pineal; CE cerebellum. Zq0294, PS_2. Bouin. Sagittal section. Hematoxylin-eosin. X 254

Fig. 7. Testis of photosensitive, nonphotostimulated Harris' sparrow with primary spermatocytes in each seminiferous tubule (Stage II of spermatogenesis). Leydig cells (arrow) are frequently seen in the interstitium. Zq0256, PS_1. Acetic acid-formalin-ethonal fixative. Hematoxylin-eosin. X 496

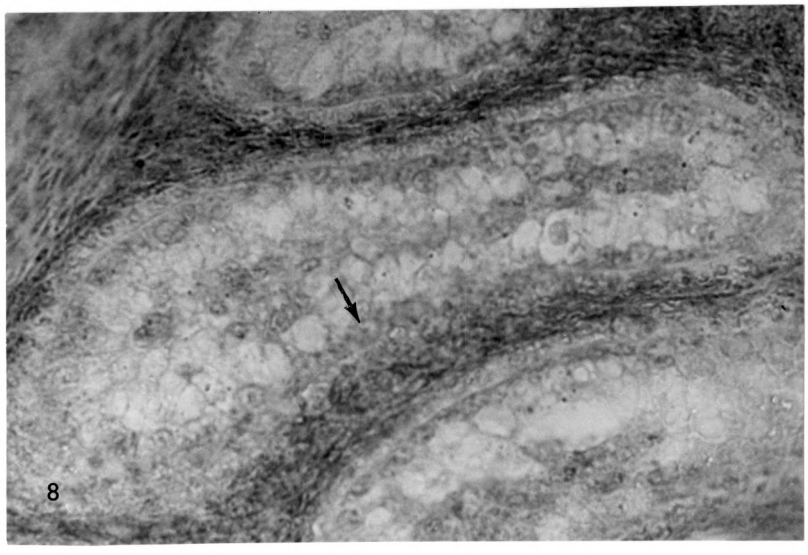
Fig. 8. Regressing testis of refractory, photostimulated Harris' sparrow. Cells in the seminiferous tubules, except for the peripheral row of spermatogonia (arrow), appear to be undergoing dissolution (Stage R). Zq0221, PLR_2. Acetic acid-formalin-ethanol fixative. Hematoxylin-eosin. X 496



6



7



8

Series PLR

Pinealectomy did not prevent the onset of testicular regression induced by continuous exposure to 20-hr daily photoperiods (Table 4). Both pinealectomized and sham-operated birds had significantly smaller testes after 99 days (group PLR_2) than after 74 days (group PLR_1) of photostimulation; testicular weights of pinealectomized birds were not significantly different from those of sham-operated birds at either killing date. Histological analysis of the testes of two pinealectomized and two sham-operated birds of group PLR_1 revealed that these testes were undergoing involution; only the basal row of spermatogonia remained intact. Leydig cells were not observed. In birds of group PLR_2, regression was advanced in four testes examined (Fig. 8), while in three others testes were in Stage I (resting condition). It is not possible to correlate either testicular stage with pinealectomy or sham operation. Among birds of group PLR_2 also, Leydig cells were not observed in the abundant interstitium. Pinealectomized birds of group PLR_1 had heavier body weights at sacrifice than sham-operated birds.

Series RL

Pinealectomy did not induce gonadal development in refractory Harris' sparrows exposed to 20-hr daily photoperiods (Table 5). Testicular weights of both sham-operated and pinealectomized birds remained small throughout the experiment. Histological examination revealed that all testes of pinealectomized and sham-operated birds were in Stage I; spermatogonia were arrayed in a single row around the periphery of the seminiferous tubule (Fig. 9). Leydig cells were not observed. Pinealectomized females showed a slight, but insignificant, increase in ovarian

Table 4. Failure of pinealectomy to inhibit the onset of testicular regression in Harris' sparrows exposed to 20-hr daily photoperiods.

Group[a]	Operational procedure[a]	Days on 20L:4D after operation[b]	Combined testicular weight[c] (mg ± S. E.)	Stage of testis[d]	Body weight at sacrifice[c] (gm ± S. E.)
PLR_1	P	54	39.60 ± 8.101 (4)[e]	R,R	39.4 ± 1.04 (4)
	S		23.28 ± 5.778 (5)[f]	R,R	35.7 ± 0.97 (5)[g]
PLR_2	P	79	6.91 ± 0.963 (4)	I,R,I	39.1 ± 1.50 (4)
	S		4.91 ± 0.473 (6)	R,I,R,R	38.5 ± 0.94 (6)

[a] Designation as in Table 1.
[b] Birds were subjected to pinealectomy or sham operation on the twentieth day of exposure to 20-hr daily photoperiods.
[c] Number of birds indicated in parentheses.
[d] Each symbol represents one bird.
[e] Significantly different ($P<0.01$) from pinealectomized birds of group PLR_2.
[f] Significantly different ($P<0.01$) from sham-operated birds of group PLR_2.
[g] Significantly different ($P<0.01$) from pinealectomized birds of group PLR_1.

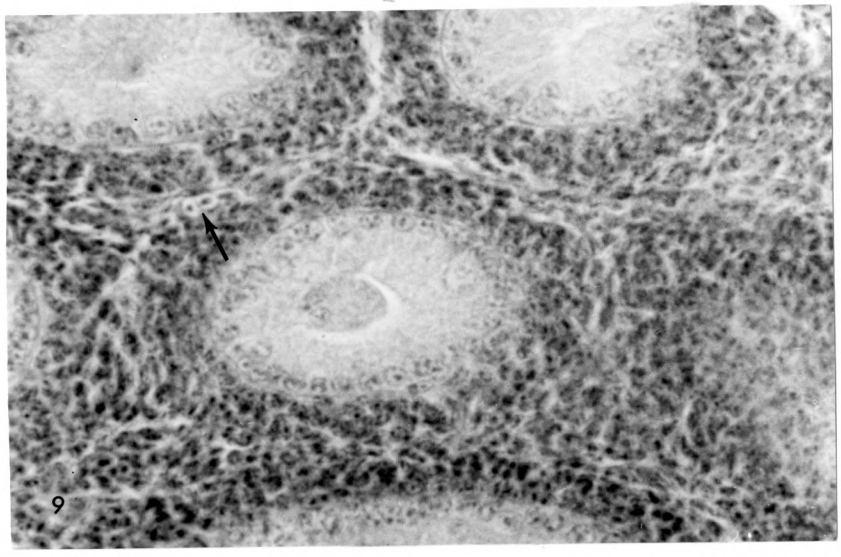
Table 5. Effect of pinealectomy on gonadal and body weight of refractory Harris' sparrows exposed to 20-hr daily photoperiods.

Group[a]	Sex	Operational procedure[a]	Months on 20L:4D after operation	Gonadal weight[b] (mg ± S. E.)	Body weight at sacrifice[c] (gm ± S. E.)
RL_1	M	P	1.5	1.97 ± 0.229 (7)	35.5 ± 1.15 (7)
		S		2.10 ± 0.020 (2)	32.8 ± 2.14 (2)
RL_2	M	P	3	2.30 ± 0.507 (5)	33.7 ± 1.84 (5)
		S		2.45 ± 0.659 (4)	34.8 ± 1.79 (4)
RL_3	F	P	1	6.44 ± 0.831 (12)[d,e]	29.1 ± 0.81 (12)
		S		4.14 ± 0.717 (5)[d,e]	29.0 ± 0.75 (5)
RL_4	F	P	2	7.12 ± 0.904 (10)[f]	30.6 ± 0.61 (10)
		S		7.58 ± 1.032 (6)	29.3 ± 1.07 (6)
RL_5	F	P	3	4.65 ± 0.604 (10)[f]	32.1 ± 0.77 (10)
		S		8.00 ± 1.310 (7)[f]	30.6 ± 2.31 (7)

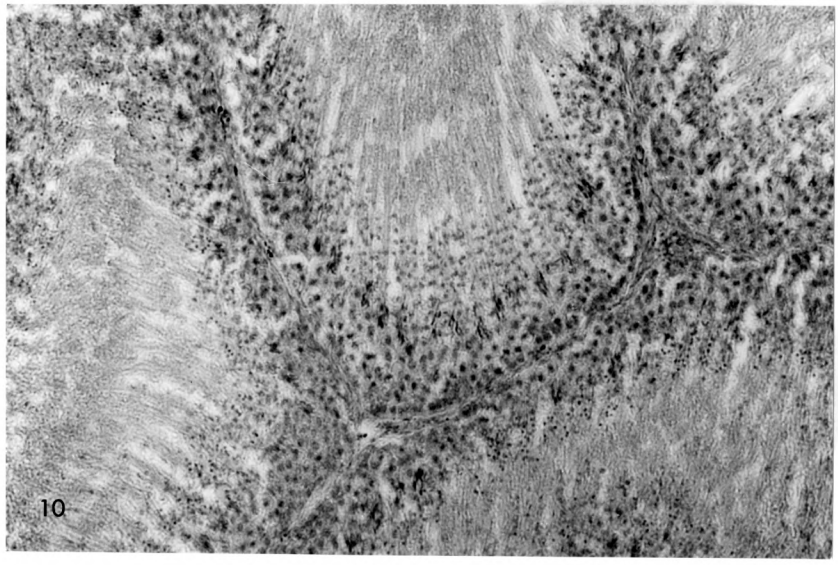
[a] Designation as in Table 1.
[b] Values for males represent combined testicular weights. Sample males had testes in Stage I of tubular development. Number of birds indicated in parentheses.
[c] Number of birds indicated in parentheses.
[d] Significantly different ($P<0.05$) from sham-operated birds of group RL_4.
[e] Significantly different ($P<0.05$) from sham-operated birds of group RL_5.
[f] Significantly different ($P<0.05$) from pinealectomized birds of group RL_5.

Fig. 9. Completely regressed testis of a refractory, photostimulated Harris' sparrow with spermatogonia in the seminiferous tubules (Stage I). Leydig cells (arrow) are occasionally seen in the abundant interstitium. Zq0204, RL_1. Acetic acid-formalin-ethanol fixative. Hematoxylin-eosin. X 496

Fig. 10. Testis of a photosensitive tree sparrow 15 days after being transferred to nonstimulatory 2-hr daily photoperiods from stimulatory 20-hr days. Regressive changes are apparent although spermatozoa are still present. Sa0881, PLS_1. Acetic acid-formalin-ethanol fixative. Hematoxylin-eosin. X 200



9



10

weight from one to two months after operation; then ovarian weight declined to a significantly lower level. However, ovaries of pinealectomized birds were not significantly smaller at three months than at one month after operation. Sham-operated females had ovaries that were significantly heavier at two and three months than at one month after operation. This gonadal development of sham-operated females coupled with the drop in ovarian weights of pinealectomized females from the second to the third month produced the only significant difference between operated and sham-operated groups.

Series PLS

Testicular weights of pinealectomized, photostimulated tree sparrows were not significantly smaller after 30 days (group PLS_2, Table 6) of exposure to 2-hr daily photoperiods than after 15 days (group PLS_1). Testicular weights of sham-operated birds decreased to significantly lower levels during this time. However, sham-operated birds experienced only a four-fold decrease in testicular weight, but weights of testes of pinealectomized birds decreased over nine-fold. Thus, the wide range of gonadal weights and the small sample size may prevent some of the seemingly apparent differences from being significant. There was no significant difference in testicular weight between pinealectomized and sham-operated birds after either 15 or 30 days of exposure to 2-hr daily photoperiods.

All five birds examined of group PLS_1 were undergoing testicular regression. One testis of a pinealectomized bird contained many spermatozoa and spermatids, but some regressive alterations were apparent (Fig. 10). Leydig cells were common in this testis, but they were not

Table 6. Failure of pinealectomy to affect testicular regression in tree sparrows exposed to 2-hr daily photoperiods.

Group[a]	Operational procedure[a]	Photoperiod during experiment[b]	Combined testicular weight[c] (mg ± S. E.)	Stage of testis[d]	Body weight at sacrifice[c] (gm ± S. E.)
PLS_1	P	20L:4D to 2L:22D	159.22 ± 48.55 (4)	R,R,R	18.1 ± 0.91 (4)
	S		60.60 ± 11.06 (4)[e]	R,R	18.8 ± 1.24 (4)
PLS_2	P	20L:4D to 2L:22D	17.51 ± 5.998 (3)	R,III	15.4 ± 1.42 (3)
	S		13.12 ± 2.956 (5)	R,R	17.3 ± 0.51 (5)

[a] Designation as in Table 2.
[b] 20L:4D for 34 days; then 2L:22D for 15 days (group PLS_1) or 2L:22D for 30 days (group PLS_2).
[c] Number of birds indicated in parentheses.
[d] Each symbol represents one bird.
[e] Significantly different ($P<0.05$) from sham-operated birds of group PLS_2.

observed in the other testes of group PLS_1. Regression was very far advanced in three testes of group PLS_2 and Leydig cells were not observed. One Stage III testis with a few Leydig cells was observed in this group.

DISCUSSION

Despite the exposed position of the avian pineal gland, there are hazards attendant to its removal. The most serious difficulty is excessive hemorrhage from one or more of the venous sinuses near the pineal. At one time, mortality from pinealectomy was high. Foà (1912) operated on 65 chickens of which 15 survived (77% mortality); Izawa (1922) pinealectomized 36 chickens, but only four survived (89% mortality). More recently, though, losses have been lower because of modifications in technique. Shellabarger and Breneman (1949) had 108 of 133 chickens survive (19% mortality); Arrington (1966) operated on 195 Japanese Quail (Coturnix coturnix japonica) and 137 survived (30% mortality). In the present study, 89 of 105 pinealectomized (82%) and 64 of 69 (92%) sham-operated Harris' and tree sparrows survived to sacrifice. Unfortunately, few investigators have recorded the mortality rates of pinealectomized and control birds. This omission would be serious if the mortality rate of epiphysectomized animals were markedly higher than that of control animals. Malm et al. (1959) reported such a difference, and also observed that body weights of epiphysectomized rats were significantly greater than those of sham-operated controls up to 14 months postoperatively. These data suggest that epiphysectomy may select for more vigorous individuals which, after postoperative recovery, may show a higher growth rate than the controls. Indeed, Izawa (1922, 1923) noted that experimental animals had a retarded growth rate for a few weeks after operation, but later grew more rapidly than controls. Thus, the frequent correlation between epiphysectomy and increase in body and organ weights may often be due to elimination of the smaller and weaker animals by operative

trauma. For the most part, there were no significant differences between body weights of pinealectomized and sham-operated birds in the results reported here. In one group (PLR_1, Table 4), body weights of pinealectomized birds were heavier than those of sham-operated birds, but unlike the results of Malm et al. (1959), there was no postoperative mortality in either operated or sham-operated groups.

Results of this study yield no evidence that the pineal affects the testes or ovaries of photosensitive, nonphotostimulated Harris' sparrows (Table 3). After retention for 5 - 10 months on 8-hr daily photoperiods, both pinealectomized and sham-operated birds exhibited gonadal weights slightly, though not significantly, larger than the minimal one to two mg weights of Harris' sparrows captured in December and January (Wilson, unpublished results). Moreover, primary spermatocytes in the seminiferous tubules of examined testes indicated tubular development had begun. These results suggest that nonphotoperiodic gonadal growth may occur during long-term retention on short days. Other investigators have noted similar results in closely related species. Golden-crowned sparrows (Zonotrichia atricapilla) retained on 10-hr daily photoperiods from mid-December had slightly enlarged testes in June and July, but testicular weights returned to a minimum by early fall (Miller, 1955). Gonads of white-throated sparrows (Zonotrichia albicollis) were advanced beyond the minimum winter condition after 8-18 months on 9-hr daily photoperiods (Weise, 1962).

Observations on birds of Series PLR and PLS (Tables 4 and 6) indicate that the pineal gland is not the primary factor contributing to testicular regression, whether induced by long-term exposure to 20-hr daily

photoperiods or by 2-hr daily photoperiods. Regression occurs independently of the pineal in both experiments. However, testes of pinealectomized birds were always slightly larger than those of sham-operated birds. Arrington (1966) made similar observations in Japanese Quail (Coturnix coturnix japonica). When switched from a 16L:8D to a 2L:22D light schedule, sexually mature C. coturnix japonica that were pinealectomized showed less testicular atrophy than controls. A large variation in individual results prevented the difference from being significant, but Arrington suggests that the pineal gland may hasten testicular atrophy.

The pineal gland is not responsible for maintaining the minimal gonadal weights that characterize refractory, photostimulated birds (Table 5). The only significant difference occurred three months after operation when pinealectomized females had smaller ovaries than sham-operated females. The significance of this observation is diminished by the wide range of apparently normal ovarian weights that both pinealectomized and sham-operated birds exhibit.

A survey of Tables 3-6 reveals that, although the results on the whole were negative, a few significant differences were scattered indiscriminately throughout. Generally, the results reported here provide little support for a role of the pineal in the reproduction of cyclic breeders. Since most investigations have utilized continuously reproducing animals, comparisons between the literature and these investigations should be made carefully.

Some of the earlier literature relating to the avian pineal gland may be discounted because of insufficient sample size for statistical analysis. Foà (1912) reported testicular hypertrophy as a result of pinealectomy in three male chickens and later (Foà, 1914) repeated the

experiment with the same results in two cocks. Izawa (1922), also working with chickens, confirmed these results with two cocks and one hen.

Disregarding these fragmentary reports, contradictions regarding the avian pineal-gonadal relationship remain in the literature. With 11 chickens, Izawa (1923) demonstrated again that pinealectomy in the first 1.5 months of life results in testicular hypertrophy between 164 and 285 days of age. On the other hand, Badertscher (1924) observed that pinealectomy in 10 males and 14 females produced no gonadal changes at 200 to 275 days of age. The data of Shellabarger and Breneman (1949) are considered among the best available (Kitay and Altschule, 1954). They found that the effects of pinealectomy at four days of age are transitory; testicular hypotrophy was noted in 19-day-old chicks, but at 42, 50 and 70 days after hatching, there is significant testicular hypertrophy. This influence of the pineal on the testes disappeared by 94 days. Later observations (Shellabarger, 1952) confirmed that, when sacrificed prior to 20 days of age, pinealectomy produces testicular hypotrophy. However, Stalsberg (1965), in extensive investigations, found no influence of pinealectomy during embryonic life on the gonads of 18- and 63-day-old chickens.

Observations of an age-variant effect of pinealectomy in the chicken (Shellabarger and Breneman, 1949; Shellabarger, 1952, 1953) and in rats (Kitay, 1954) suggest that the role of the pineal in reproduction may be significant before and during the period of sexual maturation. Accordingly, recent investigations with Japanese Quail (Coturnix coturnix japonica) have been directed to the period of sexual maturation. Pinealectomy in newly hatched quail did not prevent the gonad-inhibiting influence of 2-hr daily photoperiods (Arrington, 1966). Under stimulatory

lighting (16L:8D), five-week pinealectomized quail had slightly larger testes, but at four, six, or seven weeks no testicular or ovarian hypertrophy was noted. Sayler and Wolfson (1967), on the other hand, observed delayed ovarian development at 43 and 47 days of age in response to pineal ablation between seven and nine days of age. The results of Arrington (1966) and Sayler and Wolfson (1967), although from birds of similar age, are not directly contradictory because of sex differences. However, Homma et al. (1967) were unable to detect an effect of pinealectomy on either ovaries or testes of quail when operated at one or four weeks and sacrificed at four or seven weeks, respectively.

Two remarkable features of the gonadal response to epiphysectomy are the weakness and transient character of the effects. One tends to suspect that these characteristics could be due to severe operative trauma, as discussed above, or that they are expressions of side effects resulting from epiphyseal removal. The possibility of such a side effect resulting from hemorrhage accompanying pinealectomy is substantiated by the results of Dill (1961) who observed similar gonadal hypertrophy in immature rats after both epiphysectomy and merely cutting the midsagittal sinus. As noted previously, the pineal stalk attaches between the habenular and posterior commissures. The possibility of involvement of these two epithalamic proliferations should not be overlooked since surgical removal of the epiphysis can hardly leave them unaffected. The habenula is part of the limbic system-midbrain circuit, an integrated structural complex that includes, in addition to the habenula, the amygdala, stria terminalis, and hypothalamus (Nauta, 1963). Lesions in the amygdala (Elwers and Critchlow, 1960) and stria terminalis (Elwers and Critchlow, 1961) induce gonadal hypertrophy in immature rats. Lesions in the

archistriatum and the occipitomesencephalic tract, thought to be homologous with the amygdala and stria terminalis of mammals, induce ovarian and oviducal hypertrophy in the mallard duck (Phillips, 1964). The hypothalamus receives neural pathways from the other components of the limbic system-midbrain circuit, and the activation or inhibition of these components may directly influence the functional state of the hypothalamus. The limbic system-midbrain circuit thus provides the machinery by which the habenula, stria terminalis, and the amygdala might influence the gonads via the hypothalamus.

Consideration should be given to possible ventricular alterations resulting from epithalamic rupture. During pinealectomy, the pineal stalk apparently breaks near or at the point of attachment. The ventricular roof is extremely thin in this region; consequently, epiphysectomy may rupture the ventricular roof. Most authors have admitted considerable hemorrhage accompanying epiphysectomy and the accumulation of a hematoma in the space left by the removal of the pineal. Through ventricular rupture, alterations in the ventricular milieu may have some influence on periventricular structures, especially the hypothalamus. Increasing evidence points to the periventricular infundibular nucleus as an important component of the gonadal response mechanism of chickens (Graber et al., 1967), sparrows (Wilson, 1967), and a variety of mammals (for reviews, see Flerkò, 1963, 1966; Davidson, 1966).

As noted previously, the origin of refractoriness is unknown. Gonads of refractory birds, of some species at least, develop in response to administration of exogenous gonadotropins (Riley and Witschi, 1938; Miller, 1949; Benoit et al., 1950; Vaugien, 1954, 1955), indicating that refractoriness is not gonadal. Microscopic analysis of the testes from birds

of Group PLR_2 (Table 4) supports this interpretation. Testicular weights are approaching a minimum. While tubules of some testes are clearly undergoing involution, other testes appear to be in Stage I. Apparently, tubules are reorganized into Stage I immediately upon terminating regression but while the bird is still refractory to photostimulation. Since photosensitive birds begin testicular growth with Stage I, testes of refractory birds probably require only stimulation by the proper gonadotropin levels to initiate development. The gonadotropin content of the adenohypophysis is relatively high in refractory white-crowned sparrows (Zonotrichia leucophrys gambelii) (King et al., 1966), suggesting that the site of refractoriness must be at, or above, the level of the hypophysis; possibly it is hypothalamic.

ACKNOWLEDGMENTS

I wish to express special appreciation to Dr. F. E. Wilson for his assistance and advice in the experimental work and invaluable suggestions during the preparation of this thesis. Appreciation is also expressed to Drs. B. E. Eleftheriou, R. K. Burkhard, and D. J. Ameel for their critical reading of the manuscript.

Many people have assisted with various facets of the experimental work, and I want to thank Dr. H. T. Gier and Mr. Don J. Pihlaja for assistance with the photomicrographs; Mr. G. Roger Hands and Mr. James V. Morrison for technical assistance; and Mr. J. Kenneth Boon for his faithful assistance in capturing the sparrows used in this study. Appreciation is expressed to the many fellow graduate students who, on occasion, helped capture sparrows used in this study.

I want to express gratitude to my wife, Cecilia, for her faithful support.

This investigation was supported in part by a research grant (F370) to Dr. F. E. Wilson from the Bureau of General Research, Kansas State University, and by funds provided by the Kansas Agricultural Experiment Station, Manhattan 66502. The research was conducted while the author held an NDEA Title IV Fellowship.

REFERENCES

Andersen, D. H., and A. Wolf. 1934. Pinealectomy in rats with a critical survey of the literature. J. Physiol. (London) 81:49-62.

Arrington, L. C. 1966. Studies of the pineal function in Japanese Quail (Coturnix coturnix japonica). Ph. D. Thesis. Michigan State Univ., East Lansing, Michigan.

Badertscher, J. A. 1924. Results following the extirpation of the pineal gland in newly hatched chicks. Anat. Record 28:177-197.

Bartholomew, G. A. 1949. The effect of light intensity and day length on reproduction in the English sparrow. Bull. Museum Comp. Zool. Harvard Coll. 101:433-476.

Benoit, J., P. Mandel, F. X. Walter, and I. Assenmacher. 1950. Sensibilité testiculaire aux hormones gonadotropes hypophysaires, chez le canard domestique, au cours de la période de régression testiculaire saisonnière. Compt. Rend. Soc. Biol. 144:400-1403.

Blanchard, B. D., and M. M. Erickson. 1949. The cycle in the Gambel sparrow. Univ. Calif. (Berkeley) Publ. Zool. 47:255-318.

del Castillo, E. B. 1928. Action de la splenectomie et de l'epiphysectomie sur le cycle oestral du rat blanc. Compt. Rend. Soc. Biol. 99:1404-1405.

Davidson, J. M. 1966. Control of gonadotropin secretion in the male, p. 565-611. In L. Martini and W. F. Ganong, (ed.), Neuroendocrinology, vol. I. Academic Press, New York.

Dill, R. E. 1961. The effects of pinealectomy or pineal extracts on corticosterone secretion in the rat. (Abstr.) Anat. Record 139:222.

Donovan, C. A. 1958. Restraint and anesthesia of caged birds. Vet. Med. 53:541-543.

Elwers, M., and V. Critchlow. 1960. Precocious ovarian stimulation following hypothalamic and amygdaloid lesions in rats. Am. J. Physiol. 198:381-385.

Elwers, M., and V. Critchlow. 1961. Precocious ovarian stimulation following interruption of stria terminalis. Am. J. Physiol. 201:281-284.

Farner, D. S. 1959. Photoperiodic control of annual gonadal cycles in birds, p. 717-750. In R. B. Withrow, (ed.), Photoperiodism and related phenomena in plants and animals. AAAS Publ. 55, American Association for the Advancement of Science, Washington.

Farner, D. S., B. K. Follett, J. R. King, and M. L. Morton. 1966. A quantitative examination of ovarian growth in the white-crowned sparrow. Biol. Bull. 130:67-75.

Flerkó, B. 1963. The central nervous system and the secretion and release of luteinizing hormone and follicle stimulating hormone, p. 211-224. In A. V. Nalbandov, (ed.), Advances in neuroendocrinology. Univ. Illinois Press, Urbana.

Flerkó, B. 1966. Control of gonadotropin secretion in the female, p. 613-668. In L. Martini and W. F. Ganong, (ed.), Neuroendocrinology, vol. I. Academic Press, New York.

Foà, C. 1912. Hypertrophie des testicules et de la crete apres l'extirpation de le glande pinéale chez le coq. Arch. Ital. Biol. 57:233-252.

Foà, C. 1914. Nouvelles recherches sur la fonction de la glande pinéale. Arch. Ital. Biol. 61:79-92. Original not seen. Reported from paper by Krabbe, K. H. 1923. The pineal gland, especially in relation to the problem on its supposed significance in sexual development. Endocrinology 7:379-414

Gittes, R. F., and E. W. Chu. 1965. Reversal of the effect of pinealectomy in female rats by multiple isogeneic pineal transplants. Endocrinology 77:1061-1067.

Graber, J. W., A. I. Frankel, and A. V. Nalbandov. 1967. Hypothalamic center influencing the release of LH in the cockerel. Gen. Comp. Endocrinol. 9:187-192.

Harkins, C. E. 1937. Harris's sparrow in its winter range. Wilson Bull, 49:286-292.

Hoffman, R. A., and R. J. Reiter. 1965a. Influence of compensatory mechanisms and the pineal gland on dark-induced gonadal atrophy in male hamsters. Nature 207:658-659.

Hoffman, R. A., and R. J. Reiter. 1965b. Pineal gland: Influence on gonads of male hamsters. Science 148:1609-1611.

Hoffman, R. A., and R. J. Reiter. 1966. Responses of some endocrine organs of female hamsters to pinealectomy and light. Life Sci. 5:1147-1151.

Homma, K., L. Z. McFarland, and W. O. Wilson. 1967. Response of the reproductive organs of the Japanese quail to pinealectomy and melatonin injections. Poultry Sci. 46:314-319.

Horrax, G. 1916. Studies on the pineal gland. I. Experimental observations. Arch. Internal Med. 17:607-626.

Ifft, J. D. 1962. Effects of pinealectomy, a pineal extract and pineal grafts on light-induced prolonged estrus in rats. Endocrinology 71:181-182.

Izawa, Y. 1922. On the experimental removal of the pineal body in chickens. Trans. Soc. Pathol. Japonica 12:139-143.

Izawa, Y. 1923. Further experiments of removal of the pineal body in chickens. Trans. Soc. Pathol. Japonica 13:144-153.

Izawa, Y. 1926. On some anatomical changes which follow removal of the pineal body from both sexes of the immature albino rat. Am. J. Physiol. 77:126-139.

Kincl, F. A., and G. Benagiano. 1967. The failure of the pineal gland removal in neonatal animals to influence reproduction. Acta Endocrinol. 54:189-192.

King, J. R., B. K. Follett, D. S. Farner, and M. L. Morton. 1966. Annual gonadal cycles and pituitary gonadotropins in Zonotrichia leucophrys gambelii. Condor 68:476-487.

Kitay, J. I. 1954. Effects of pinealectomy on ovary weight in immature rats. Endocrinology 54:114-116.

Kitay, J. I., and M. D. Altschule. 1954. The pineal gland: a review of the physiologic literature. Harvard Univ. Press, Cambridge. 271 p.

Krabbe, K. H. 1955. Development of the pineal organ and a rudimentary parietal eye in some birds. J. Comp. Neurol. 103:139-149.

Lerner, A. B., J. D. Case, Y. Takahashi, T. H. Lee, and W. Mori. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. J. Amer. Chem. Soc. 80:2587.

Malm, O. J., O. E. Skaug, and P. Lingjaerde. 1959. The effect of pinealectomy on bodily growth, survival rate and P32 uptake in the rat. Acta Endocrinol. 30:22-28.

Meyer, C. J., R. J. Wurtman, M. D. Altschule, and E. A. Lazo-Wasem. 1961. The arrest of prolonged estrus in "middle-aged" rats by pineal gland extract. Endocrinology 68:795-800.

Miller, A. H. 1949. Potentiality for testicular recrudescence during the annual refractory period of the golden-crowned sparrow. Science 109:546.

Miller, A. H. 1955. The expression of innate reproductive rhythm under conditions of winter lighting. Auk 72:260-264.

Moszkowska, A. 1963. L'antagonisme epiphysohypophysaire. Ann. Endocrinol. (Paris) 24:215-226.

Nauta, W. J. H. 1963. Central nervous organization and the endocrine motor system, p. 5-21. In A. V. Nalbandov, (ed.), Advances in neuroendocrinology. Univ. Illinois Press, Urbana.

Phillips, R. E. 1964. "Wildness" in the mallard duck: Effects of brain lesions and stimulation on "escape behavior" and reproduction. J. Comp. Neurol. 122:139-155.

Quay, W. B. 1965. Indole derivatives of pineal and related neural and retinal tissues. Pharmacol. Rev. 17:321-345.

Quay, W. B., and A. Renzoni. 1967. I rapporti diencefalici e la variabilita nella duplice struttura del complesso epifisario degli uccelli. The diencephalic relations and variably bipartite structure of the avian pineal complex. (Bilingual edition). Riv. Biol. 60:9-75.

Reiss, M., R. H. Davis, M. B. Sideman, I. Mauer, and E. S. Plichta. 1963. Action of pineal extracts on the gonads and their function. J. Endocrinol. 27:107-118.

Reiter, R. J. 1967. The effect of pineal grafts, pinealectomy, and denervation of the pineal gland on the reproductive organs of male hamsters. Neuroendocrinology 2:138-146.

Reiter, R. J., and R. J. Hester. 1966. Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation of the endocrine systems of hamsters. Endocrinology 79:1168-1170.

Reiter, R. J., R. A. Hoffman, and R. J. Hester. 1966a. The role of the pineal gland and of environmental lighting in the regulation of the endocrine and reproductive system of rodents. Edgewood Arsenal Tech. Rept. (EATR 4032), Medical Research Laboratory, US Army Edgewood Arsenal, Md. 51 p.

Reiter, R. J., R. A. Hoffman, and R. J. Hester. 1966b. The effects of thiourea, photoperiod and the pineal gland on the thyroid, adrenal and reproductive organs of female hamsters. J. Exp. Zool. 162:263-268.

Renton, A. D., and H. W. Rusbridge. 1932. Late effects of pinealectomy in rats. Proc. Soc. Exp. Biol. Med. 30:766.

Riley, G. M., and E. Witschi. 1938. Comparative effects of light stimulation and administration of gonadotropic hormones on female sparrows. Endocrinology 23:618-624.

Roth, W. D. 1965. Metabolic and morphologic studies on the rat pineal organ during puberty, p. 552-563. In Structure and function of the epiphysis cerebri, J. A. Kappers and J. P. Schade, (ed.), Progress in brain research, vol. 10. Elsevier Publ Co., Amsterdam.

Sayler, A., and A. Wolfson. 1967. Avian pineal gland: progonadotropic response in the Japanese quail. Science 158:1478-1479.

Shellabarger, C. J. 1952. Pinealectomy vs. pineal injection in the young cockerel. Endocrinology 51:152-154.

Shellabarger, C. J. 1953. Observations of the pineal in the White Leghorn capon and cockerel. Poultry Sci. 32:189-197.

Shellabarger, C. J., and W. R. Breneman. 1949. The effects of pinealectomy on young White Leghorn cockerels. Proc. Indiana Acad. Sci. 59:299-302.

Simonnet, H., and L. Thieblot. 1951. Recherches experimentales sur la physiologie de la glande pineale. Acta Endocrinol. 7:306-320.

Stalsberg, H. 1965. Effects of extirpation of the epiphysis cerebri in 6-day chick embryos. Acta Endocrinol. Suppl. 97:1-119.

Stevens, O. A. 1957. Fall migration and weather, with special reference to Harris' sparrow. Wilson Bull. 69:352-359.

Swenk, M. H., and O. A. Stevens. 1929. Harris's sparrow and the study of it by trapping. Wilson Bull. 41:129-177.

Tilney, F., and L. F. Warren. 1919. The morphology and evolutionary significance of the pineal body. Am. Anat. Mem. 9:1-257.

Vaugien, L. 1954. Influence de l'obscuration temporaire sur la durée de la phase réfractaire du cycle sexuel du moineau domestique. Bull. Biol. France et Belg. 88:294-309.

Vaugien, L. 1955. Sur les réactions ovariennes du moineau domestique soumis, durant le repos sexuel, a des injections de gonadotrophine sérique de jument gravide. Bull. Biol. France et Belg. 89:1-15.

Weise, C. M. 1962. Migratory and gonadal responses of birds on long-continued short daylengths. Auk 79:161-172.

Wilson, F. E. 1965. The effects of hypothalamic lesions on the photoperiodic testicular response in white-crowned sparrows, Zonotrichia leucophrys gambelii. Ph. D. Thesis. Washington State Univ., Pullman.

Wilson, F. E. 1967. The tubero-infundibular neuron system: a component of the photoperiodic control mechanism of the white-crowned sparrow, Zonotrichia leucophrys gambelii. Z. Zellforsch. Mikroskop. Anat. 82:1-24.

Wilson, F. E. 1968. Testicular growth in Harris' sparrow (Zonotrichia querula). Auk (in press).

Wilson, F. E., and G. R. Hands. 1968. Hypothalamic neurosecretion and photoinduced testicular growth in the tree sparrow, Spizella arborea. Z. Zellforsch. Mikroskp. Anat. (in press).

Wragg, L. E. 1967. Effects of pinealectomy in the newborn female rat. Am. J. Anat. 120:391-402.

Wurtman, R. J., M. D. Altschule, and U. Holmgren. 1959. Effects of pinealectomy and of a bovine pineal extract in rats. Am. J. Physiol. 197:108-110.

Wurtman, R. J., and J. Axelrod. 1965. The formation, metabolism, and physiologic effects of melatonin in mammals, p. 520-529. In Structure and function of the epiphysis cerebri, J. A. Kappers and J. P. Schadé, (ed.), Progress in brain research, vol. 10. Elsevier Publ. Co., Amsterdam.

Wurtman, R. J., J. Axelrod, and E. W. Chu. 1963. Melatonin, a pineal substance: Effect on the rat ovary. Science 141:277-278.

Wurtman, R. J., W. Roth, M. D. Altschule, and J. J. Wurtman. 1961. Interactions of the pineal and exposure to continuous light on organ weights of female rats. Acta Endocrinol. 36:617-624.

Ziesmer, C. 1951. Eine Verbesserung der Silber-Imprägnierung nach Bodian. Z. Wiss. Mikroskopie 60:57-59.

FAILURE OF PINEALECTOMY TO ALTER GONADAL ACTIVITY IN HARRIS' SPARROW (ZONOTRICHIA QUERULA) AND THE TREE SPARROW (SPIZELLA ARBOREA)

by

RICHARD S. DONHAM

B. S., Kansas State University, 1965

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Although evidence pertaining to the function of the pineal body in birds and mammals is inconclusive, an antigonadotropic role is often indicated. Pinealectomy frequently results in gonadal hypertrophy in the laboratory rat and domestic fowl; in addition, melatonin, an indole derivative that has been isolated from the pineal has an antigonadotropic effect in the rat. In contrast to these continuously breeding animals, cyclic breeders have received little attention in pineal-gonadal studies. If the gonads of cyclic breeders are influenced by the pineal, then pinealectomy may result in conspicuous alterations in the gonadal cycle. Events of gonadal cycles of migratory passerine species are easily studied since they may be precisely controlled by manipulation of the daily photoperiod. This study was designed to assess the influence of the pineal on the gonadal cycles of male and female Harris' sparrows (Zonotrichia querula) and tree sparrows (Spizella arborea).

To determine the influence of the pineal on the quiescent gonads of photosensitive, nonphotostimulated birds, pinealectomy was performed on male and female Harris' sparrows retained on 8-hr daily photoperiods. Autopsy 1.5 - 3 months after removal of the pineal did not reveal any testicular or ovarian changes. Two experiments were designed to study the influence of pinealectomy during testicular regression. In the first, testicular regression was induced in Harris' sparrows by continued exposure to 20-hr daily photoperiods. Pinealectomy did not significantly alter the course of testicular regression. In the second experiment, testicular regression was induced in tree sparrows by transferring photostimulated, photosensitive birds to nonstimulatory 2-hr daily photoperiods. In this experiment also, testicular regression progressed normally in pinealectomized birds. In both experiments studying regression, testes

of pinealectomized birds were slightly, but not significantly, larger than those of sham-operated birds. Thus, there is some indication that further investigations of the pineal-gonadal relationship during the onset of gonadal regression may be fruitful. Finally, an experiment studied the influence of pinealectomy on the gonads of Harris' sparrows made refractory by exposure to long daily photoperiods for several months. Pinealectomy did not induce testicular or ovarian hypertrophy in refractory birds exposed to 20-hr daily photoperiods.

These observations do not generally support an hypothesis that assigns to the pineal an antigonadotropic role. Rather, they suggest that the avian pineal body does not significantly affect the gonadal cycle.